



## Subtle shifts in microbial communities occur alongside the release of carbon induced by drought and rewetting in contrasting peatland ecosystems

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1 Subtle shifts in microbial communities occur  
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4 ecosystems

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## 34 **Abstract**

35 Peat represents a globally significant pool of sequestered carbon. However, peatland carbon  
36 stocks are highly threatened by anthropogenic climate change, including drought, which leads  
37 to a large release of carbon dioxide. Although the enzymatic mechanisms underlying  
38 drought-driven carbon release are well documented, the effect of drought on peatland  
39 microbial communities has been little studied. Here, we carried out a replicated and  
40 controlled drought manipulation using intact peat ‘mesocosm cores’ taken from bog and fen  
41 habitats, and used a combination of community fingerprinting and sequencing of marker  
42 genes to identify community changes associated with drought. Community composition  
43 varied with habitat and depth. Moreover, community differences between mesocosm cores  
44 were stronger than the effect of the drought treatment, emphasising the importance of  
45 replication in microbial marker gene studies. While the effect of drought on the overall  
46 composition of prokaryotic and eukaryotic communities was weak, a subset of the microbial  
47 community did change in relative abundance, especially in the fen habitat at 5 cm depth.  
48 ‘Drought-responsive’ OTUs were disproportionately drawn from the phyla *Bacteroidetes* and  
49 *Proteobacteria*. Collectively, the data provide insights into the microbial community changes  
50 occurring alongside drought-driven carbon release from peatlands, and suggest a number of  
51 novel avenues for future research.

## 52 **Introduction**

53 Anthropogenic climate change is one of the key issues of the 21<sup>st</sup> century, with the potential  
54 to severely impact human lives as well as natural ecosystems<sup>1</sup>. The effect of climate change  
55 on soil biodiversity and consequent ecological processes is of particular concern because of  
56 the potential for detrimental positive feedback effects. An overarching concern is that  
57 warming can lead to an increase in soil respiration and consequently an increased release of  
58 carbon dioxide into the atmosphere<sup>2-5</sup>. Micro-organisms should be considered when

59 attempting to understand and predict the effects of climate change on soil processes, since  
60 microbial communities are central to the decomposition of soil organic matter<sup>6</sup> and are  
61 directly responsible for a large proportion of soil respiration<sup>7</sup>. In addition, microbial  
62 communities play a key role in determining gas fluxes<sup>8</sup> and rates of nutrient cycling<sup>9</sup>. While it  
63 is difficult to separate effects which are mediated by changes to soil microbial communities  
64 from the direct effects of environmental change, there is strong evidence that the soil  
65 microbial community is important in determining the way that soil processes respond to  
66 environmental change<sup>10-12</sup>.

67 Peat soils are an extremely important global store of carbon: estimates for the total amount of  
68 carbon stored in Northern peatlands vary from 273 Gt C to 547 Gt C<sup>13,14</sup>. However, climate  
69 change represents a serious threat to temperate peatlands; for example, within the UK the  
70 area covered by blanket peat is projected to decline, with the potential for peatlands to change  
71 from carbon sinks to carbon sources<sup>15</sup>. Likewise, the amount of carbon stored within  
72 peatlands in Canada<sup>16</sup>, the USA<sup>17</sup> and across the Northern hemisphere<sup>18</sup> is also predicted to  
73 decline.

74 The effect of drought on carbon cycling within peatlands has been an area of particular  
75 interest. While climate change models project an increase in total precipitation at high  
76 latitudes, rainfall is likely to become more concentrated in extreme events interspersed with  
77 periods of dry weather<sup>19</sup>, while higher temperatures will increase water loss from soils<sup>20</sup>.  
78 Together, these effects will lead to an increase in the likelihood of drought events<sup>19,20</sup> and a  
79 fall in peatland summer water table<sup>21,22</sup>. Unlike drier habitats, where drought leads to  
80 moisture-limiting conditions and a reduction in carbon release by heterotrophic micro-  
81 organisms<sup>6</sup>, drought in peatlands facilitates the aeration of previously anaerobic peat layers.  
82 Aeration stimulates microbial decomposition, and consequently leads to increased carbon  
83 dioxide release<sup>23</sup>. The effect of drought on peatland carbon dioxide fluxes often outlasts the

84 duration of the drought itself by a considerable margin due to the degradation of inhibitory  
85 phenolic compounds under anaerobic conditions<sup>23</sup>. Therefore, the effects of increased  
86 summer drought frequency on peatland carbon fluxes represent a potential positive feedback  
87 loop, with the potential to accelerate rates of global warming. There is some evidence that the  
88 composition of peatland microbial communities responds to long-term water table changes<sup>24-</sup>  
89 <sup>26</sup>, with Actinobacteria and fungi responding particularly strongly after several years of water  
90 table drawdown<sup>24</sup>. Microbial community composition also changes in response to short-term  
91 drought<sup>27-29</sup>, although the exact microbial groups involved remain unclear. Moreover, while  
92 protozoa have been neglected in modern-day studies of drought effects on peatland microbial  
93 communities, paleoecological studies indicate that testate amoebae community composition  
94 in peat is strongly influenced by water table depth<sup>30</sup>. Nevertheless, the microbial mechanisms  
95 underlying drought-driven carbon dioxide release from peat remain poorly understood.

96 The development of high-throughput sequencing-based approaches for the identification of  
97 microorganisms has provided an unprecedented opportunity to advance our understanding of  
98 microbial communities in natural environments, and to explore the effects of environmental  
99 change on these communities. Initial DNA-based microbial ecology studies were limited by  
100 the low throughput of existing sequencing methodologies or the low resolution of  
101 ‘community fingerprinting’ approaches<sup>31</sup>, but the introduction of high-throughput sequencing  
102 platforms immediately decreased the cost per base pair of sequencing data. Lower sequencing  
103 costs and paradigm shifts in throughput have enabled sequencing of rRNA genes to be used  
104 on much broader scales and across a wide spectrum of biological diversity<sup>32</sup>.

105 In order to identify changes in microbial communities which occur concurrently to the release  
106 of carbon from peat ecosystems, here we aimed to use high-throughput marker gene  
107 sequencing to identify the proportion of the microbial biosphere which is affected by drought  
108 and rewetting in bogs and fens, two habitats which are representative of the majority of

109 temperate peatlands in the Northern hemisphere. A replicated and controlled drought  
110 manipulation was carried out using peat ‘mesocosm cores’ collected from both habitats (Fig.  
111 1). In addition to the concurrent monitoring of greenhouse gas fluxes, DNA was extracted  
112 and purified from two contrasting depths below the peat surface. Extracted DNA was then  
113 subjected to automated ribosomal intergenic spacer analysis (ARISA), a community  
114 fingerprinting technique enabling rapid and low-cost estimation of diversity within a  
115 microbial community, in order to confirm that drought affected microbial communities.  
116 ARISA fingerprinting was followed by sequencing, bioinformatics and statistical analysis of  
117 16S and 18S rRNA genes to obtain a more detailed perspective of community changes.

## 118 **Results**

119 ARISA fingerprinting of bacterial communities yielded bands ranging in size from 110-2839  
120 bp, while ARISA fingerprinting of fungal communities yielded bands ranging from 54-2851  
121 bp. Binning of ARISA amplicons into 5 bp bins gave a total of 185 bins for bacterial  
122 communities and 87 for fungal communities.

123 Sequencing yielded a total of 102,439,895 and 104,156,662 paired-end reads for 16S and 18S  
124 rRNA genes, respectively. Of the 16S rRNA gene reads, 29,337,117 passed quality control  
125 steps and were clustered into 49,892 OTUs. Of the 18S rRNA gene reads, a total of  
126 17,214,346 passed quality control and paired-end joining, which were clustered into 43,058  
127 OTUs. Following standardisation of read numbers, rarefaction curves were generated to  
128 assess sequencing coverage (Fig. S1) and these suggested that sequencing coverage was  
129 adequate, particularly for samples from the bog.

### 130 **Effects of Habitat and Depth on Microbial Community**

131 NMDS ordination of ARISA fingerprinting data showed some separation of samples taken  
132 from bog mesocosm cores at 20 cm depth from other habitats and depths (Fig. 2a). Fungal  
133 communities were more weakly affected by habitat and depth, but samples taken from the

134 bog at 5 cm appeared to be distinct from all other samples on the third axis (Fig. 2b).  
135 PERMANOVA tests confirmed that ARISA fingerprinting profiles of both bacterial and  
136 fungal communities were significantly affected by habitat (bacteria:  $P=0.001$ ; fungi:  
137  $P=0.001$ ; Table S1) and depth (bacteria:  $P=0.001$ ; fungi:  $P=0.001$ ; Table S1) although in  
138 each case the effect size ( $R^2$  value) was small (Table S1), indicating that habitat and depth  
139 only accounted for a small proportion of overall variation. Bacterial communities were also  
140 significantly affected by the interaction between habitat and depth ( $P=0.001$ ; Table S1).

141 Sequencing of 16S and 18S rRNA genes identified an effect of habitat and depth on both  
142 prokaryotic (16S) and eukaryotic (18S) communities that was stronger than that detected by  
143 ARISA fingerprinting. For both markers, samples clustered by habitat along the first axis and  
144 by depth along the second axis (Fig. 2c; 2d). PERMANOVA tests confirmed that there were  
145 significant effects of habitat (16S:  $P=0.001$ ; 18S:  $P=0.001$ ; Table S2), depth (16S:  $P=0.001$ ;  
146 18S:  $P=0.001$ ; Table S2) and the interaction term (16S:  $P=0.001$ ; 18S:  $P=0.001$ ; Table S2) on  
147 community composition.

148 Seven prokaryotic phyla and six eukaryotic phyla each made up  $>1\%$  of reads in at least one  
149 habitat and depth (Fig. 3a; 3b). *Acidobacteria* and *Proteobacteria* contributed by far the  
150 highest proportions of prokaryotic reads in both the bog and the fen: *Acidobacteria*  
151 contributed 47% of reads in the bog but only 13% in the fen, while *Proteobacteria* contributed  
152 20% of reads in the bog and 19% in the fen. However, a large proportion of prokaryotic  
153 OTUs could not be assigned to phylum level at the requisite *utax* confidence level of 0.85,  
154 with fen communities containing a higher proportion of 'Unassigned' OTUs than bog  
155 communities.

156 Within the eukaryotic communities, an even higher proportion of the community could not be  
157 assigned. In particular, at 20 cm depth 90% of reads belonged to OTUS which could not be

158 assigned to phylum level at the chosen confidence level (0.85). Amongst OTUs which could  
159 be assigned, the highest numbers of reads were contributed by Chloroplastida (green plants;  
160 9% of reads in the bog and 3% in the fen) and Fungi (11% of reads in the bog and 6% in the  
161 fen).

162 Linear mixed effect models were fitted to transformed proportional abundances of reads from  
163 the most abundant phyla in order to determine which factors affected phylum-level  
164 community composition. Of the seven prokaryotic phyla which made up >1% of the  
165 community, all but *Verrucomicrobia* were significantly affected by habitat and depth, and all  
166 were significantly affected by the interaction between habitat and depth (Table S3). In  
167 particular, *Acidobacteria* made up a higher proportion of reads in the bog and at 5 cm depth;  
168 *Proteobacteria* made up the highest proportion of reads in the bog at 5 cm and the lowest in  
169 the bog at 20 cm; and *Bacteroidetes* made up the highest proportion of reads in the fen and at  
170 5 cm depth (Fig. 3a). Conversely, three of the six eukaryotic phyla tested were significantly  
171 affected by habitat (Alveolata, Stramenopiles, Rhizaria), four were affected by depth (Fungi,  
172 Alveolata, Metazoa, Rhizaria), and four were affected by the interaction between habitat and  
173 depth (Fungi, Alveolata, Stramenopiles, Rhizaria; Table S4). Reads assigned to phyla  
174 Alveolata, Rhizaria and Stramenopiles were all more abundant in the fen than the bog. Reads  
175 assigned to Fungi, Alveolata and Rhizaria were each more abundant at 5 cm than 20 cm.

#### 176 Effect of Drought and Environmental Variables on Microbial Communities

177 Under drought conditions and during rewetting, treated mesocosm cores had significantly  
178 higher redox potentials and significantly lower water content than control mesocosm cores  
179 (Fig. 4d; Fig. S2; Table S5). Carbon dioxide fluxes rose significantly during drought but  
180 returned to control levels during rewetting, while methane fluxes fell and remained  
181 suppressed throughout the rewetting period (Fig. 4a; 4b; Table S6). The concentration of  
182 dissolved organic carbon (DOC) was significantly lower in fen mesocosm cores than in bog

183 mesocosm cores, and was lower in droughted mesocosm cores (pre-drought measurements of  
184 DOC concentration were not taken; Fig. 4c; Table S6). However, there was also an  
185 unexpected rise in the water content of the peat between the first two sampling time points  
186 (Fig. S2). There was a significant effect of treatment on bacterial ARISA fingerprinting  
187 profiles in the bog at both depths and in the fen at 20 cm (Table S7), while the effect of  
188 treatment on the fungal community was only significant in the fen at 5 cm. There was a  
189 significant two-way interaction between time point and treatment on fungal communities in  
190 the fen at 20 cm. In addition, prokaryotic communities at 20 cm in both habitats changed  
191 significantly between sampling time points (Table S7) and on fungal communities in the bog  
192 at both depths and in the fen at 20 cm (Table S7). However, sequencing of 16S and 18S  
193 rRNA genes indicated that there was no effect of the drought-rewetting treatment on overall  
194 community composition. NMDS ordinations of these communities indicated that the  
195 mesocosm core from which samples were taken had a stronger effect on community  
196 composition than time point or treatment (Fig. 5). PERMANOVA tests confirmed this  
197 observation: while community composition was significantly different between treatments,  
198 neither time point nor the interaction effect had a significant effect (Table S8) and therefore  
199 the treatment effect observed in sequencing data was likely due to pre-existing differences  
200 between the mesocosm cores assigned to each treatment (Fig. 5). Conversely, the effect of  
201 core was strongly significant in all habitats and depths and for both markers (Table S9).  
202 Application of envfit confirmed differences in microbial communities between mesocosm  
203 cores, and also found significant correlations between vegetation and the prokaryotic  
204 community (Table 1; Fig. 5). Prokaryotic community composition was significantly  
205 correlated to CO<sub>2</sub> fluxes in the bog at 5 cm depth and the fen at 20 cm depth (although  
206 significance was marginal in the latter case), while methane fluxes were not significantly  
207 correlated to community composition (Table 1). Fewer significant correlations existed

208 between environmental variables and the community composition of microbial eukaryotes,  
209 although there was a weak correlation between eukaryotic community composition and the  
210 concentration of phenolic compounds in both habitats at 20 cm depth (Table 1).

211 None of the seven most abundant prokaryotic phyla showed significant changes in relative  
212 abundance in response to drought (Table S3). Of the six most eukaryotic phyla, only the  
213 relative abundance of Rhizaria was significantly affected by drought, showing an increase in  
214 abundance when the water table reached its minimum in the fen at 5 cm depth before falling  
215 again during rewetting (interaction between time point and treatment:  $F_{8,177}=2.6$ ,  $P=0.009$ ;  
216 Fig. 6; Table S4).

217 Following abundance filtering of all OTUs, linear mixed effect models were fitted in order to  
218 detect OTUs which were significantly affected by the interaction between time point and  
219 treatment (hereafter ‘drought-affected OTUs’). Drought-affected OTUs are summarised in  
220 Table 2, and full details given in Tables S10-S13 and Figs. S3-S6. Briefly, far more drought-  
221 affected OTUs were detected in the fen at 5 cm than in any other habitat and depth; in the fen  
222 at 5 cm, 37 prokaryotic OTUs and 7 eukaryotic OTUs showed significant changes in relative  
223 abundance during drought. Conversely, the number of drought-affected prokaryotic OTUs in  
224 other habitats and depths ranged from 2-5 OTUs, while the number of drought affected  
225 eukaryotic OTUs ranged from 1-3. NMDS ordination of only drought-affected OTUs  
226 confirmed that the effect of drought was most consistent in the fen at 5 cm (Fig. S7).

227 Amongst drought-affected OTUs in the fen at 5 cm, the phyla *Proteobacteria* and  
228 *Bacteroidetes* were overrepresented relative to their abundance in the dataset as a whole:  
229 *Proteobacteria* made up 27% of the overall community and 41% of drought-affected OTUs,  
230 while *Bacteroidetes* made up only 7% of the overall community but 39% of drought-affected  
231 OTUs. The majority of the drought-affected OTUs which were assigned to *Bacteroidetes*

232 showed a negative response to drought while the majority of those assigned to *Proteobacteria*  
233 responded positively, but there were exceptions to this pattern. Few OTUs could be assigned  
234 to genus level, but negatively drought-affected OTUs included likely members of genera  
235 *Paludibacter* and *Geobacter* while positively drought-affected OTUs included members of  
236 genera *Massalia*, *Duganella* and *Caulobacter*. Eukaryotic drought-affected OTUs in the fen  
237 at 5 cm contained members of the Alveolata, Rhizaria and Nematoda, as well as four OTUs  
238 which could not be assigned at phylum level (Table 2).

239 Very few drought-affected OTUs occurred in the other habitats. From the 16S rRNA gene  
240 dataset, there were five drought-affected OTUs in the fen at 20 cm depth, four in the bog at 5  
241 cm and two in the bog at 20 cm depth. Amongst these, *Acidobacteria* and Unassigned  
242 *Bacteria* were the most common taxonomic assignments (Table 2).

## 243 **Discussion**

244 While differences in microbial community composition between habitats and depths were  
245 detected in analyses based on both ARISA fingerprinting and amplicon sequencing data, the  
246 effect of habitat and depth was much stronger when community analysis was based on  
247 sequencing data (Fig. 2). The greater resolution in SSU rRNA sequencing data likely results  
248 from the limitations of ARISA fingerprinting, which is based on intraspecies differences in  
249 the length of the intergenic spacer region of ribosomal rRNA genes. However, in highly  
250 diverse environments such as soils, multiple species can share the same intergenic spacer  
251 length<sup>31</sup>, reducing the resolution of this technique.

252 The phylum-level composition of microbial communities in both habitats was similar to  
253 previous studies of peat soils<sup>33-35</sup>, suggesting that the composition of peatland communities is  
254 conserved across geographically disparate regions, at least at the level of phylum. Relative  
255 abundances of all abundant bacterial phyla were significantly affected by both habitat and  
256 depth, while only a subset of eukaryotic phyla exhibited demonstrable differences in

257 community composition between habitats and depths. However, phyla containing microbial  
258 eukaryotes (Fungi, Stramenopiles, Rhizaria and Alveolata) were more strongly affected by  
259 habitat and depth than were macrofaunal phyla, likely because the methods used were not  
260 designed to detect variations in the abundance of macrofaunal organisms. Additionally, the  
261 large proportion of eukaryotic reads belonging to OTUs which could not be annotated to  
262 phylum level likely made differences in abundance more difficult to detect. The strong effect  
263 of habitat on the relative abundance of many phyla is unsurprising given that almost all  
264 measured environmental variables differed between the two habitats; in comparison to the fen  
265 mesocosm cores, bog cores had lower mean pH values and redox potentials, but much higher  
266 concentrations of DOC.

267 Within each habitat and depth, there were significant differences in the community  
268 composition of the mesocosm cores, potentially linked to differences in environmental  
269 variables between different cores. In particular, the percentage cover of different plant  
270 functional groups was significantly correlated to microbial community composition in several  
271 cases, as were the concentration of phenolic compounds and the pH of the peat. Plants are an  
272 important driver of microbial communities and are able to influence the rhizosphere  
273 microbiome directly, for example *via* root exudates<sup>36</sup>. Alternatively, plant communities can  
274 act as more effective indicators of soil chemistry over longer time periods, compared to  
275 insights derived from a single snapshot in time of microbial community composition<sup>37</sup>.

276 As expected, the drought treatment led to a rise in redox potential and a corresponding release  
277 of carbon dioxide while both methane flux and the concentration of DOC fell, corresponding  
278 to the results of previous studies<sup>21,23</sup>. However, unlike previous studies, carbon dioxide fluxes  
279 in in droughted mesocosm cores immediately returned to similar levels as observed in control  
280 cores when rewetting began, despite the fact that the redox potential remained elevated. The  
281 fall in carbon dioxide flux as the water table rises may result from carbon dioxide dissolving

282 in the porewater rather than being released at the surface of the peat; the concentration of  
283 dissolved inorganic carbon (DIC) increases rapidly on rewetting<sup>38</sup>, suggesting the potential  
284 for porewater to absorb the gases released by microbial metabolism. Alternatively, carbon  
285 dioxide release due to increased respiration by autotrophs during drought cannot be ruled out;  
286 in some cases, root respiration increases following aeration of peat<sup>38</sup>. Unexpectedly, the water  
287 content of the peat rose between the first and second time points in all habitats and at all  
288 depths (Fig. S2). The reasons for this rise are unclear as the mesocosm cores were transferred  
289 to bins of water within hours of collection, with small holes drilled for water exchange.  
290 However, the mesocosm cores in the current experiment were larger than those used in  
291 previous studies<sup>39</sup>, creating a potential mechanism for less efficient water exchange between  
292 cores and the surrounding water.

293 Despite the clear effect of drought and rewetting on carbon cycling, the effect of the drought-  
294 rewet treatment on microbial community composition was weak and overshadowed by  
295 differences between mesocosm cores. ARISA fingerprinting suggested a significant, albeit  
296 weak, effect of drought within certain depths and habitats, but there was no corresponding  
297 effect in the sequence-based analysis. This discrepancy may have arisen as a result of  
298 differences in the lengths of amplicons measured by each method: ARISA amplicons were  
299 165-1,580bp long, while sequenced rRNA amplicons were 300-350bp. In freshwater lakes,  
300 seasonal changes in community composition derived from analysis of invertebrate  
301 environmental DNA have been more rapidly detected when analysing smaller amplicons<sup>40</sup>, as  
302 the size distribution of DNA becomes more skewed towards smaller fragments over time<sup>41</sup>.

303 The weak response of microbial communities to drought and rewetting in both datasets  
304 suggests that the increased carbon dioxide flux observed during drought was not mediated by  
305 changes in microbial community composition. In addition, CO<sub>2</sub> fluxes were only significantly  
306 correlated to prokaryotic community composition in two of the four possible combinations of

307 habitat and depth (Table 1), and in both cases the correlation was weak (Fig. 5). However, it  
308 is possible that members of the microbial community changed in activity rather than  
309 abundance, or that genuine community changes were obscured by DNA belonging to  
310 dormant or dead organisms<sup>42,43</sup>. Although not feasible in the present study,  
311 metatranscriptomic analyses would further clarify the relative contributions of shifts in the  
312 active versus the overall community to drought<sup>44</sup>. While metatranscriptomic analysis has yet  
313 to be applied to temperate peatlands, in permafrost peatlands metatranscriptomic analysis  
314 gives subtly different results to metagenomics, and so it is likely that differences exist  
315 between the active community and the DNA present in soil<sup>45</sup>. In addition, awareness of the  
316 role played by rare species in community function and response to environmental change has  
317 recently begun to increase<sup>46,47</sup>; due to the difficulties in separating genuinely rare OTUs from  
318 erroneous reads, rare OTUs were not the focus of this study, but it is possible that future  
319 studies could gain new insights by focusing on the rare portion of the microbial biosphere in  
320 peat ecosystems.

321 While amplicon sequencing suggested that drought and rewetting did not affect overall  
322 community composition, there were nonetheless indications that individual groups of micro-  
323 organisms responded to the treatment. In particular, phylum Rhizaria (a phylum of protists)  
324 made up a significantly higher proportion of the community in the fen at 5 cm at minimum  
325 water table (Fig. 6). The response of Rhizaria to drought is of potential interest, as protists  
326 may play important roles in mediating the response of environmental processes to  
327 environmental change. For example, grazing by ciliates may determine the rate of change in  
328 bacterial biomass under warming conditions<sup>48</sup> while a fall in the abundance of mixotrophic  
329 testate amoeba led to a rise in peatland carbon dioxide emissions following warming<sup>49</sup>.  
330 Rhizaria also play an important role in the export of carbon from marine planktonic

331 systems<sup>50</sup>. The role played by protists (especially Rhizaria) in the context of bottom up and  
332 top down controls in the carbon cycle of droughted peatlands therefore merits further study.

333 Testing for significant effects of the drought-rewet treatment on individual prokaryotic OTUs  
334 revealed that the relative abundance of a number of OTUs changed relative to control  
335 conditions during drought and/or rewetting, particularly in the fen at 5 cm depth. A large  
336 proportion of ‘drought-affected OTUs’ in the fen at 5 cm depth belonged to *Bacteroidetes* and  
337 *Proteobacteria*. Notably, both of these phyla have been previously identified as containing a  
338 high proportion of non-dormant cells when compared to other bacterial phyla<sup>43</sup>, potentially  
339 meaning that they more rapidly respond to environmental change by increasing or decreasing  
340 in abundance rather than activity. Only two negatively drought-affected OTUs could be  
341 assigned to genus level: one of these belonged to genus *Paludibacter*, the sole described  
342 member of which is an obligately anaerobic fermenter<sup>51</sup>, and the other to *Geobacter*, a genus  
343 of metal-reducers. Therefore, a number of obligate anaerobes may fall in abundance in the  
344 active layer of fens following drought. Patterns were more difficult to detect amongst the  
345 positively drought-affected OTUs, many of which belonged to the *Proteobacteria*, a diverse  
346 phylum containing a broad range of functional categories<sup>52</sup>. Intriguingly, two positively  
347 drought-affected OTUs were affiliated with taxa that are commonly associated with  
348 petroleum-contaminated soils: genus *Caulobacter* and family *Sphingomonadaceae*<sup>53,54</sup>. Both  
349 taxa contain aerobic bacteria, prompting speculation that aeration during drought may allow  
350 proliferation of bacteria involved in aerobic degradation of organic matter. However, it should  
351 be noted that few were significant following the application of corrections for multiple  
352 comparisons and thus this analysis should be viewed as a hypothesis-generating rather than a  
353 confirmatory study.

354 Collectively, the current study highlights an array of important insights into the microbial  
355 mechanisms underpinning the drought-driven release of carbon from globally important peat

356 ecosystems. The replicated design and enhanced taxonomic resolution afforded by the marker  
357 gene analyses demonstrated marked heterogeneity between putatively similar experimental  
358 cores. Furthermore, the study suggests that drought-driven changes in carbon fluxes in  
359 peatland ecosystems are not associated with large-scale community changes, and thus raises  
360 the possibility that these changes may be caused by shifts in the activity rather than the  
361 composition of the microbial community or may be a result of small shifts in beta diversity  
362 which have large effects on community function. We predict that future combinations of  
363 metagenomic and metatranscriptomic analyses will yield further insights to complement  
364 existing theories and highlight biogeochemical mechanisms that could be targeted to enhance  
365 carbon retention in globally important peat ecosystems.

## 366 **Materials and Methods**

### 367 **Collection of Mesocosm Cores and Experimental Design**

368 Mesocosm cores were collected from two sites representing typical temperate bog and fen  
369 habitats. Fen cores were extracted from Cors Erddreiniog, a low-lying fen in mid-Anglesey,  
370 North Wales, UK (grid reference SH461826), which is designated a Special Area of  
371 Conservation and represents a nationally important area of alkaline and calcareous fen habitat  
372 (JNCC 2007). Bog cores were taken from Marchlyn Mawr (NVC classification M6 [*Carex*  
373 *echinata* – *Sphagnum recurvum/auriculatum* mire]<sup>55</sup>), on the outskirts of Snowdonia National  
374 park (grid reference SH610625). Marchlyn Mawr was chosen because of its proximity to  
375 important drinking water reservoirs.

376 Peat ‘mesocosm cores’ were collected in lengths of PVC pipe (each 20 cm in diameter and 35  
377 cm in length), following a protocol adapted from that of Freeman, Lock and Reynolds<sup>39</sup>.  
378 After collection, mesocosm cores were kept in a controlled temperature room at 8-10 °C for  
379 the duration of the experiment and lit by fluorescent daylight tubes (mean PAR: 305.4  $\mu\text{mol}$   
380  $\text{m}^{-2} \text{sec}^{-1}$ ) on a 16:8 hour day-night cycle. Cores were placed in bins which were filled to the

381 level of the peat surface with artificial rainwater for bog cores and artificial groundwater for  
382 fen cores, with holes drilled near the base of each core to allow water exchange with the  
383 surrounding water. The composition of the rainwater followed a standard recipe<sup>56</sup>, while the  
384 groundwater was produced following a custom recipe that emulated the chemical  
385 composition of groundwater at Cors Erddreiniog according to earlier measurements (Table  
386 S14).

387 Within each habitat, five of the ten mesocosm cores were randomly assigned to the drought-  
388 rewet treatment while the remaining five acted as controls. The water table in the control  
389 cores was level with the surface of the peat throughout, mimicking field conditions, while the  
390 water table in drought cores at each sampling time point is described in Table S15 and in Fig.  
391 1. Briefly, the first two time points were simulated as ‘pre-drought’, during which the water  
392 table in each mesocosm core was level with the peat surface. Following the pre-drought, the  
393 water table in treatment cores was gradually lowered over nine weeks, kept stable at 20 cm  
394 below the peat surface for six weeks, and rewetted over six weeks (Table S15). The length  
395 and intensity of the drought treatment was based on a natural drought which occurred in 2006  
396 in the Cerrig-yr-Wyn catchment in mid-Wales<sup>23</sup>. Additionally, all mesocosm cores were  
397 allowed to acclimatise in the controlled temperature room for approximately one month prior  
398 to the first sampling time point.

#### 399 Sample Collection and DNA Extraction

400 Soil and gas samples were collected at three week intervals (Table S15). Gas samples were  
401 taken between 10am and noon following methods previously used to analyse carbon fluxes  
402 from peat mesocosm cores<sup>23</sup>. Briefly, a sealed headspace was placed over each mesocosm  
403 core. A 20 cm<sup>3</sup> gas sample was removed from the headspace at 0, 15, 30, 60 and 120 minutes  
404 and injected into an evacuated 12 ml glass vial (Labco Medical Supplies). Gas samples were  
405 analysed on a Varian 450-GC fitted with a flame ionisation detector (FID) and a methaniser.

406 At each time point, the machine was calibrated using three gas mixtures of known  
407 concentration obtained from Scientific and Technical Gases Ltd (Newcastle under Lyme,  
408 Staffordshire, UK). In each case, a linear regression line was calculated between time and  
409 carbon dioxide concentration and the slope of the regression was taken as the average flux  
410 value.

411 Subsequently, six gram samples of peat were collected at 5 cm (chosen to correspond to the  
412 most biogeochemically active layer of the peat) and 20 cm (chosen to correspond to  
413 minimum water table) below the peat surface. Immediately after the removal of soil samples,  
414 the redox potential of the peat was measured using a redox probe with an Ag/AgCl reference  
415 electrode in 3 M KCl. To adjust the value obtained to the 'true' value (i.e. that which would  
416 have been obtained using a standard hydrogen electrode), a correction factor of +207 was  
417 added prior to further analysis<sup>57</sup>. The hole from which peat samples were taken were  
418 immediately plugged to prevent water loss and destruction of the peat structure. The size of  
419 the cores was sufficient that each sampling event removed only a small proportion of the total  
420 material, and no subsistence of mesocosm cores was observed over the course of the  
421 experiment.

422 The soil samples were homogenised thoroughly using flame-sterilised tools before DNA was  
423 extracted from a 0.25 g subsample using a MoBio PowerSoil kit (Cambio, Cambridge),  
424 following manufacturer's instructions. Following preliminary tests, DNA extracted with the  
425 MoBio PowerSoil kit was found to contain lower levels of PCR inhibitors than alternative  
426 methods. DNA was eluted into 100  $\mu$ L sterile Tris-EDTA buffer (10 mM Tris, 1 mM EDTA,  
427 pH 7.6) and stored at -80 °C prior to further analysis. Samples were further purified using a  
428 MoBio PowerClean kit following manufacturer's instructions, as purification was found to  
429 result in more consistent PCR amplification during downstream molecular biological  
430 manipulation.

431 Percentage water content of peat was measured by weighing a subsample of peat before and  
432 after drying at 108 °C for 48 hours. Phenol oxidase activity was assayed using the phenolic  
433 amino acid L-3,4- dihydroxy phenylalanine (L-DOPA) as a substrate, as described in detail  
434 by Dunn *et al.*<sup>58</sup>. The concentration of phenolic compounds was assayed using Folin-  
435 Ciocalteu Reagent<sup>59</sup>: briefly, a 1 cm<sup>3</sup> subsample of peat was taken using a cut-off syringe and  
436 weighed. Water-soluble phenolics were extracted by homogenising the peat subsample with 9  
437 ml of water before centrifuging the resulting slurry. 250 µl of supernatant was added to each  
438 of three wells of a clear microplate and baseline absorbance measured prior to addition of  
439 12.5 µl Folin-Ciocalteu reagent and 37.5 µl filtered sodium carbonate solution (200 mg l<sup>-1</sup>).  
440 Samples were mixed, incubated at room temperature for 90 minutes, and absorbance  
441 measured at 750 nm. A calibration curve was produced using dilutions of phenol solution in  
442 the range of 0-10 mg l<sup>-1</sup>. A pH meter was inserted into peat slurry (1 g peat: 9 ml water) in  
443 order to measure the pH of the peat. DOC measurement was carried out using a Thermalox  
444 TOC/TN analyser equipped with a non-dispersive infrared CO<sub>2</sub> detector.

#### 445 ARISA Fingerprinting

446 Automated ribosomal intergenic spacer analysis (ARISA) is a community fingerprinting  
447 technique enabling rapid and low-cost estimation of diversity within a microbial community.  
448 The method involves amplifying the intergenic spacer region of microbial ribosomal DNA  
449 and analysing the length of the obtained amplicons. The length of the intergenic spacer region  
450 is very variable, and amplicons of different sizes are therefore expected to represent separate  
451 species or strains<sup>31</sup>. Although all cores were used in biogeochemical analyses (N=5), this was  
452 not possible for nucleic acid-based analyses, and so within each combination of time point  
453 and treatment a subset of three of these mesocosm cores were selected for ARISA  
454 fingerprinting and all downstream molecular genetic work. Within each chosen core, ARISA

455 fingerprinting was carried out on samples taken from both depths and all nine time points,  
456 giving a total of 216 samples for this part of the analysis.

457 The primers employed for ARISA of bacterial communities were ITSF (5'-  
458 GTCGTAACAAGGTAGCCGTA-3') and ITSReub (5'-GCCAAGGCATCCACC-3'), which  
459 have been shown to outperform other commonly used ARISA primers<sup>60</sup>. As there was no  
460 existing comparison of primer pairs for ARISA of fungal communities, selected primers were  
461 tested using Primer Prospector software<sup>61</sup>. Based on this comparison, a combination of  
462 ITS1WH (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-  
463 TCCTCCGCTTATTGATATGC-3') was selected.

464 Each ARISA PCR reaction contained 9.45 µl of nuclease-free water, 12.5 µl of PCR Master  
465 Mix (Promega), 1 µl of each primer (10 µM), 0.05 µl molecular grade bovine serum albumin  
466 (1mg/ml, Thermo Scientific) and 1 µl template DNA (diluted to 10 ng/µl) to give a final  
467 volume of 25 µl. For ARISA fingerprinting of bacterial communities, thermal cycling  
468 parameters were 95 °C for 2 minutes for initial denaturation, followed by thirty cycles of 95  
469 °C for one minute (denaturation), 52 °C for 45 seconds (annealing), 72 °C for 1.5 minutes  
470 (extension), and a final extension period of five minutes. An annealing temperature of 54.2  
471 °C was used for the fungal ARISA primers, with all other steps in the PCR program identical  
472 to those for the bacterial communities. PCR amplicon lengths were measured on a Qiaxcel  
473 Advanced (Qiagen), using a Qiaxcel High Resolution kit and method OM1200  
474 (recommended by the manufacturer for amplicon lengths between 0.5 and 1.5 kbp).

#### 475 Sequencing of 16S and 18S rRNA Genes

476 As with ARISA fingerprinting, sequencing of rRNA genes could only be carried out for  
477 samples taken from a subset of mesocosm cores: for consistency, the same three mesocosm  
478 cores were chosen for sequencing as for ARISA fingerprinting. Library preparation,

479 sequencing of 16S and 18S rRNA genes and initial quality control was carried out by the  
480 Earth Microbiome Project<sup>62</sup> (<http://www.earthmicrobiome.org/>) according to standard  
481 protocols. Briefly, the V4 region of the 16S rRNA gene was amplified using primers 515f  
482 (5'-GTGCCAGCMGCCGCGGTAA-3') and 806r (5'-GGACTACHVGGGTWTCTAAT-  
483 3')<sup>63</sup>, which amplify both bacterial and archaeal sequences, and the V9 region of the 18S  
484 rDNA gene was amplified using Illumina\_Euk\_1391f (5'-GTACACACCGCCCGTC-3') and  
485 Illumina\_EukBr (5'-TGATCCTTCTGCAGGTTACCTAC-3')<sup>64</sup>. Sequencing was carried  
486 out on an Illumina HiSeq in rapid run mode, giving paired-end reads of 150bp in length.  
487 Quality control and demultiplexing was carried out in QIITA (<http://qiita.microbio.me/>), a  
488 QIIME-based repository and analysis platform for “-omics” data, and was equivalent to  
489 quality control in QIIME using default parameters.

#### 490 Sequence Processing and operational taxonomic unit (OTU) Clustering

491 Following removal of poor quality reads by the Earth Microbiome Project, further quality  
492 control and OTU clustering was carried out in VSEARCH<sup>65</sup>, a method which has been  
493 proven to output high quality OTUs<sup>66</sup>, followed by taxonomic assignment using USEARCH  
494 v8.12<sup>67</sup>. VSEARCH was run on the HPC Wales system. Identical reads were merged and *de*  
495 *novo* chimera prediction was carried out using UCHIME, as implemented in VSEARCH,  
496 with default parameters. Next, chimeras were manually removed and OTUs were clustered  
497 within VSEARCH at 97% similarity, and an OTU table suitable for downstream analysis was  
498 generated using the script ‘uc2otutab.py’ ([http://drive5.com/python/uc2otutab\\_py.html](http://drive5.com/python/uc2otutab_py.html)).  
499 Taxonomy was assigned to each OTU using the ‘utax’ command in USEARCH v8.12<sup>67</sup>.  
500 Taxonomy was assigned against the provided UTX reference data for 16S rRNA genes,  
501 which is based on RDP training set v15<sup>68</sup>, and against the SILVA database v111 for 18S  
502 rRNA genes<sup>69</sup>.

503 Where large differences in read numbers exist between samples and differences between  
504 treatments are subtle, rarefaction has been shown to perform outperform other methods of  
505 normalization prior to clustering analyses (e.g. NMDS ordination)<sup>71</sup>. Thus, prior to further  
506 analysis, read numbers were standardised in all samples using the ‘rrarefy’ command from  
507 the ‘VEGAN’ package<sup>70</sup>. Samples in the 16S rRNA gene dataset were standardised to contain  
508 70,000 reads each and samples in the 18S rRNA gene dataset were standardised to contain  
509 20,000 reads each. The thresholds used for standardisation were chosen to include the  
510 majority of samples, but exclude samples where sequencing had failed. Samples which  
511 contained fewer reads than these thresholds were removed from the dataset: 10 samples were  
512 removed from the 16S rRNA gene dataset and 9 from the 18S rRNA gene dataset as they did  
513 not contain the requisite number of reads for the read number standardisation step.

#### 514 Statistical Analyses

515 The experimental design gave rise to four independent variables: habitat, depth, treatment and  
516 time point. To test for significant effects of these variables on fluxes of carbon dioxide (CO<sub>2</sub>)  
517 and methane (CH<sub>4</sub>) and on the concentration of dissolved organic carbon (DOC), linear  
518 mixed effect models were fitted using package ‘nlme’ in R<sup>72</sup>. Linear mixed effect models are  
519 widely applicable in ecological analyses<sup>73</sup>, and were required in this case to allow for the  
520 effect of mesocosm core. Since multiple samples were taken from each mesocosm core,  
521 analyses would otherwise have been confounded by temporal pseudoreplication. Model  
522 selection was based on the recommendations of Zuur *et al.*<sup>73</sup>. Briefly, models were initially  
523 fitted with all main effects (habitat, depth, treatment, and time point) and all two and three-  
524 way interactions included. Mesocosm core was included as a random effect. Interaction  
525 effects were removed sequentially based on hypothesis testing using a likelihood ratio test  
526 until only significant interactions remained (with the exception of the interaction between

527 time point and treatment, which was kept in all models due to the importance of this term to  
528 the focal aims of the study).

529 Community data from ARISA fingerprinting was analysed using the ‘vegan’ package in R<sup>70</sup>.  
530 First, fragment sizes were sorted into 5bp bins and converted to presence-absence data. Next,  
531 NMDS ordination was carried out on Jaccard distances across all samples (appropriate for  
532 binary (presence-absence) data) followed by PERMANOVA tests.

533 Following standardisation of sequence numbers across samples, OTU abundance tables were  
534 subject to the same multivariate analyses as ARISA fingerprinting data, but based on Bray-  
535 Curtis dissimilarity rather than binary Jaccard distances, due to the semi-quantitative  
536 information included in this kind of data<sup>74</sup>. In order to focus on the community composition  
537 of microbial eukaryotes, all OTUs in the 18S rRNA dataset which were assigned to phyla  
538 Holozoa, Chloroplastida and Metazoa (at any confidence level) were excluded from  
539 calculations of NMDS ordinations and PERMANOVA tests. Unassigned OTUs were also  
540 removed. Results of NMDS ordination were linked to environmental variables using function  
541 ‘envfit’ from package ‘VEGAN’<sup>70</sup>: this was done within habitat-depth subsets due to the  
542 strong effect of habitat and depth on both microbial community composition and  
543 environmental variables. Envfit first calculates the direction of the effect of a given variable:  
544 for ‘vectors’ (continuous variables) this is done by calculating the direction of maximum  
545 correlation between the variable and the ordination scores, while for ‘factors’ (discrete  
546 variables) envfit calculates the average ordination score for each factor level. Next,  
547 significance values are calculated for each variable using a permutation test.

548 To test for significant effects of habitat, depth, treatment and time point on the most abundant  
549 phyla (i.e. those which made up >1% of the community), the proportion of each phylum was  
550 logit-transformed<sup>75</sup> and linear mixed effect models were fitted as described above for gas  
551 fluxes.

552 Linear mixed effect models were also used to identify individual OTUs that showed  
553 significant responses to drought. Within each habitat-depth combination, OTUs were first  
554 filtered to include only those OTUs which were sufficiently abundant (at least 1 read per  
555 1000 reads in one sample) and present in at least 20% of samples. This strict filtering was  
556 carried out in order to minimise effects of rare OTUs; the high proportion of rare OTUs in the  
557 dataset was considered likely to generate spurious results. Following filtering, the relative  
558 abundance of each OTU was logit transformed<sup>75</sup> and linear mixed effect models were fitted  
559 with mesocosm core as a random effect (random intercept model). Benjamini-Hochberg  
560 corrections<sup>76</sup> were calculated to correct for the large number of comparisons. Where a  
561 significant effect of the interaction between time point and treatment was found, OTU  
562 abundances were carefully scrutinised and cases where the interaction effect was due to  
563 outlier effects were removed.

564

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756

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## 762 **Author Contributions**

763 All authors reviewed the manuscript. CP performed the experiment and analysed the data.  
764 SC, CF, NF, JM and PG conceived of the experiment and contributed to the discussion of  
765 results. TJ, LM & AE contributed DOC data and analysis. GA carried out the sequencing and  
766 initial quality control of sequencing data. CP wrote the manuscript, and all authors  
767 contributed to review of the manuscript.

## 768 **Competing Financial Interests**

769 We are not aware of any competing financial interests.

## 770 **Data Accessibility**

771 Sequence data is publically archived on the ENA/EBI database (accession  
772 number ERP016584) and on QIITA (<https://qiita.ucsd.edu/>; study ID:10278).

773

774 **Table 1** Results of ‘envfit’ applied to ordination of microbial communities within each  
 775 habitat-depth subset. Significant p-values are denoted by \* (p < 0.05), \*\* (p < 0.01), and \*\*\*  
 776 (p < 0.001). OTUs assigned to the following phyla were excluded from the 18S rRNA dataset  
 777 prior to analysis: Holozoa, Metazoa, Chloroplastida and ‘NA’. Phenol=concentration of  
 778 phenolic compounds; P-Ox=phenol oxidase activity; %Moss=percentage cover of mosses;  
 779 %Grasses=percentage cover of graminoids; %Shrubs=percentage cover of shrubs;  
 780 CO<sub>2</sub>=carbon dioxide flux; CH<sub>4</sub>=methane flux.

781

Marker	Variable	Bog- 5cm		Bog- 20cm		Fen- 5cm		Fen-20cm	
		R <sup>2</sup>	p						
<b>16S rRNA gene</b>	<b>Core</b>	0.71	0.001**	0.27	0.002**	0.07	0.2	0.14	0.04*
	<b>pH</b>	0.02	0.6	0.03	0.5	0.36	0.001**	0.12	0.045
	<b>Phenol</b>	<0.01	0.9	0.03	0.5	0.22	0.005**	0.35	0.001**
	<b>P-Ox</b>	0.14	0.024*	0.17	0.01*	0.02	0.6	0.05	0.3
	<b>%Moss</b>	0.23	0.002**	0.41	0.001**	0.08	0.1	0.23	0.002**
	<b>%Grasses</b>	0.17	0.017*	0.30	0.001**	0.20	0.006**	0.50	0.001**
	<b>%Shrubs</b>	0.59	0.001**	0.11	0.052	NA	NA	NA	NA
	<b>CO<sub>2</sub></b>	0.12	0.048*	0.11	0.058	0.02	0.6	0.15	0.02*
	<b>CH<sub>4</sub></b>	0.04	0.3	0.0174	0.6	0.04	0.4	0.07	0.2
<b>18S rRNA gene</b>	<b>Core</b>	0.04	0.4	<0.01	0.8	0.05	0.3	0.17	0.008**
	<b>pH</b>	<0.01	1	<0.01	0.9	0.01	0.7	0.27	0.001**
	<b>Phenol</b>	0.11	0.07	0.2	0.005**	0.10	0.06	0.20	0.006**
	<b>P-Ox</b>	0.05	0.3	0.04	0.4	0.05	0.3	0.19	0.009**
	<b>%Moss</b>	<0.01	0.8	0.01	0.7	0.08	0.1	0.27	0.001
	<b>%Grasses</b>	0.02	0.7	0.05	0.3	<0.01	0.9	0.03	0.5
	<b>%Shrubs</b>	0.05	0.3	0.08	0.1	NA	NA	NA	NA
	<b>CO<sub>2</sub></b>	0.03	0.4	0.01	0.8	0.04	0.4	0.08	0.10
	<b>CH<sub>4</sub></b>	<0.01	0.8	0.05	0.3	0.05	0.3	<0.01	0.9

782

783 **Table 2** Summary of the number and taxonomic affiliation of significantly drought-affected  
 784 OTUs in sequencing datasets from each habitat and at each depth. Drought-affected OTUs  
 785 shown were significantly affected by the treatment: time point interaction effect at a p-value  
 786 of <0.05 prior to the application of corrections for multiple comparisons. Only taxonomic  
 787 annotations with a utax confidence value of >0.85 are included, with annotations at lower  
 788 confidence values classed as ‘unassigned’.

<b>Marker</b>	<b>Habitat</b>	<b>Positive Effect</b>	<b>Negative Effect</b>
<b>16S rRNA gene</b>	<b>Bog-5cm</b>	Proteobacteria (1); Acidobacteria (2); Bacteroidetes (1)	None
	<b>Bog-20cm</b>	Acidobacteria (2)	None
	<b>Fen-5cm</b>	Acidobacteria (1); Bacteroidetes (2); Proteobacteria (11); Unassigned Bacteria (3)	Pacearchaeota (1); Bacteroidetes (12); Firmicutes (1); Proteobacteria (4); Unassigned Bacteria (2)
	<b>Fen-20cm</b>	Acidobacteria (1); Unassigned Bacteria (1)	Unassigned Bacteria (3)
<b>18S rRNA gene</b>	<b>Bog-5cm</b>	Rhizaria (2); Unassigned Eukaryote (1)	None
	<b>Bog-20cm</b>	None	None
	<b>Fen-5cm</b>	Alveolata (1); Nematoda (1); Rhizaria (1); Unassigned Eukaryote (2)	Unassigned Eukaryote (2)
	<b>Fen-20cm</b>	Strameopiles (1)	None

789

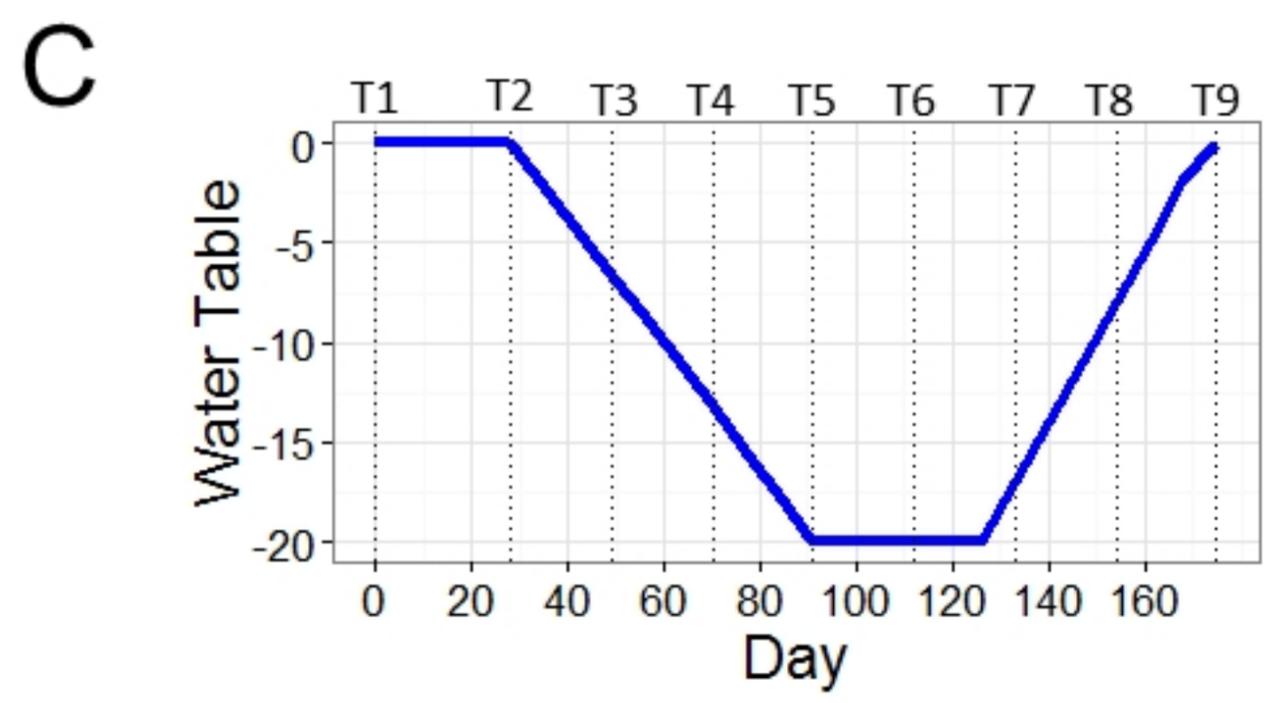
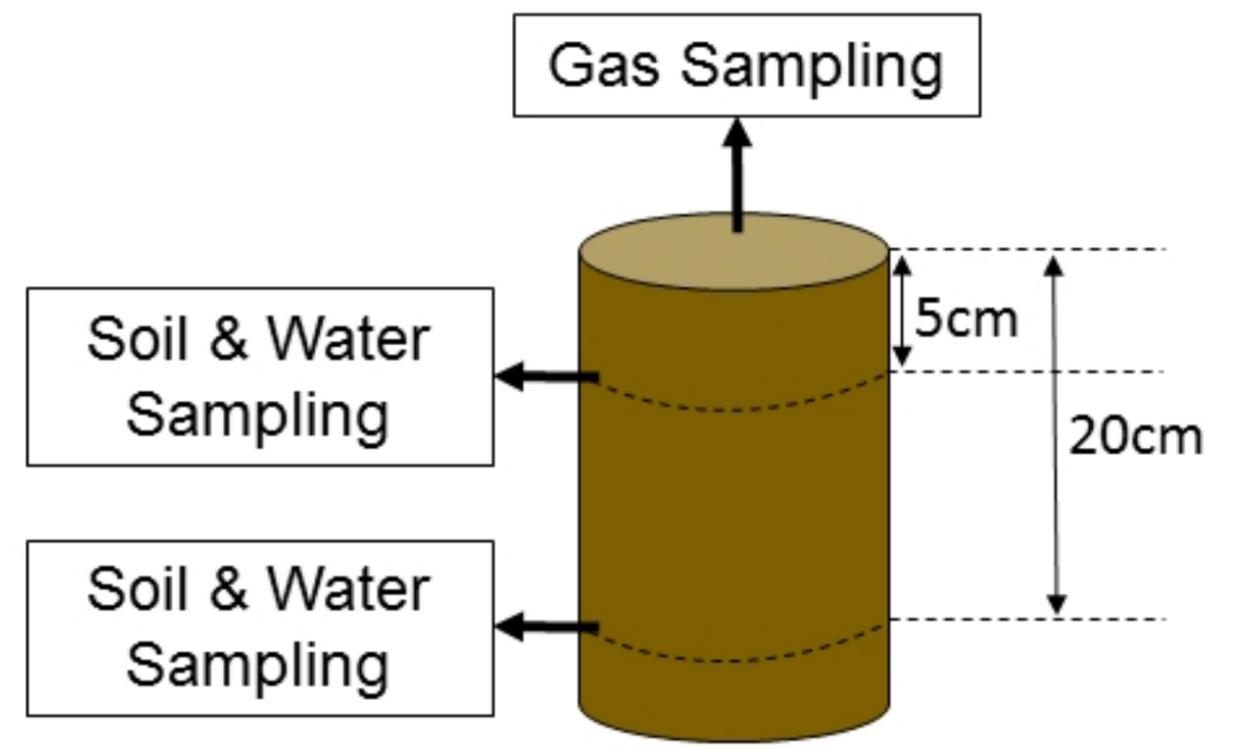
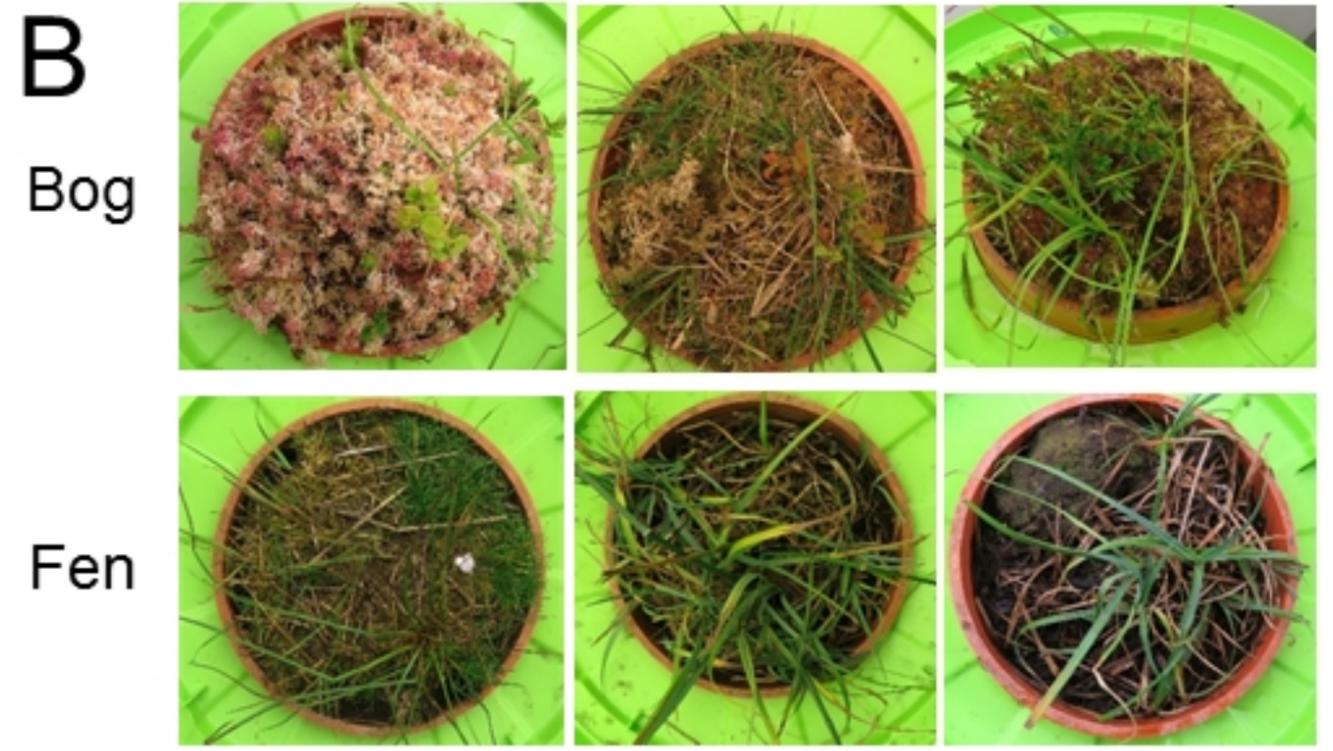
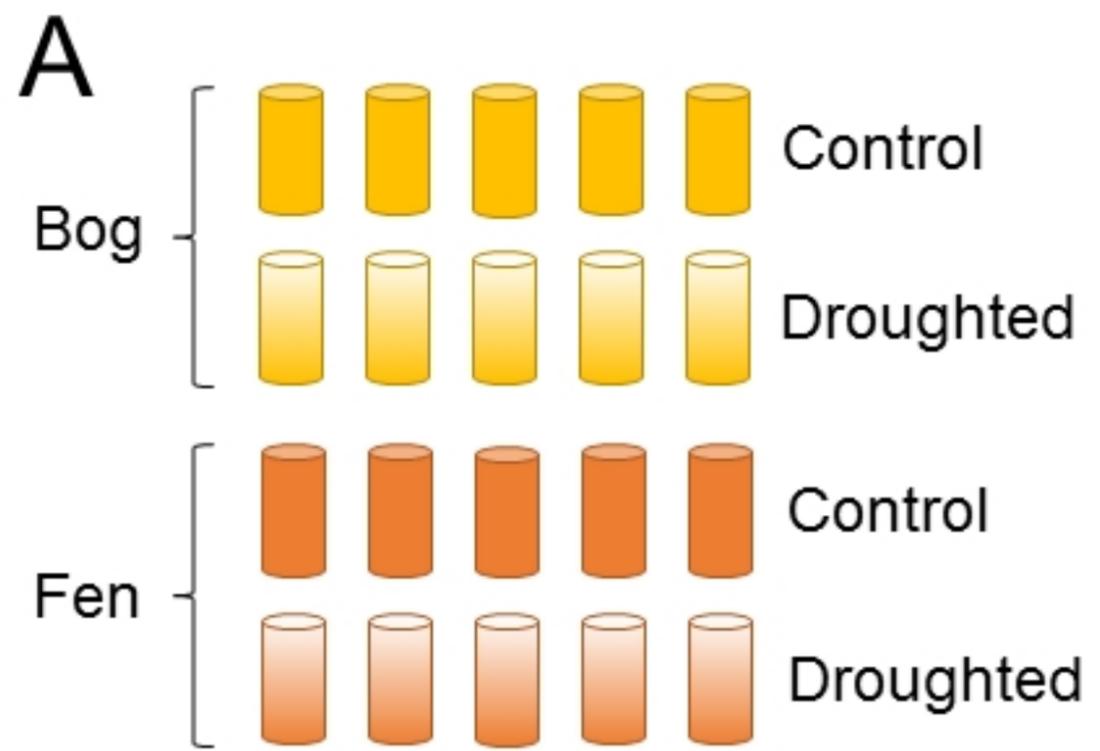
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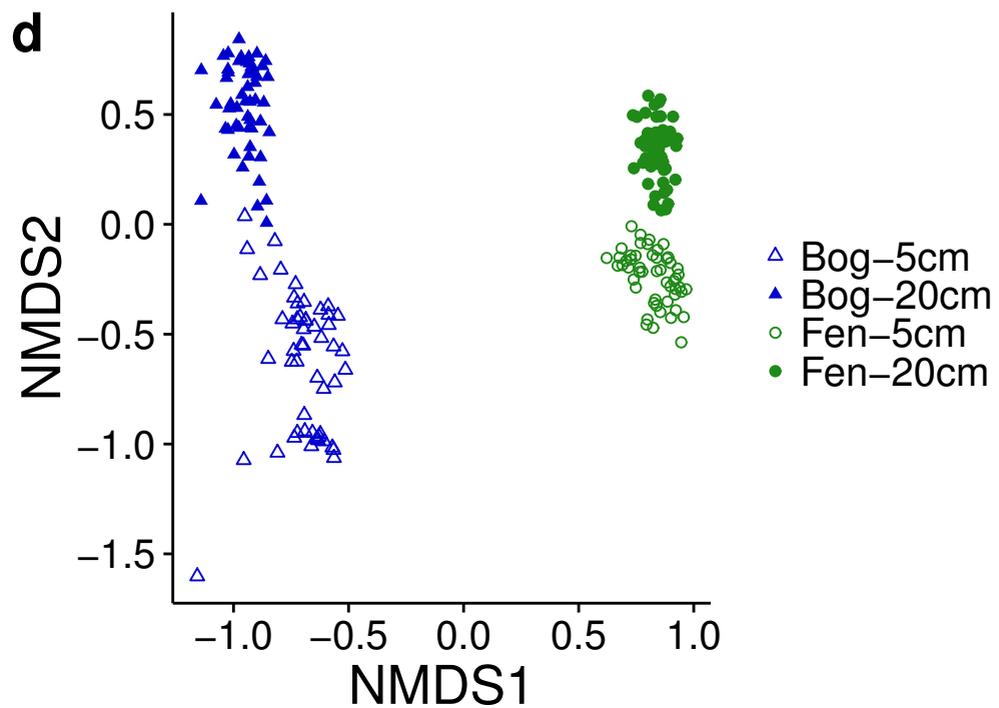
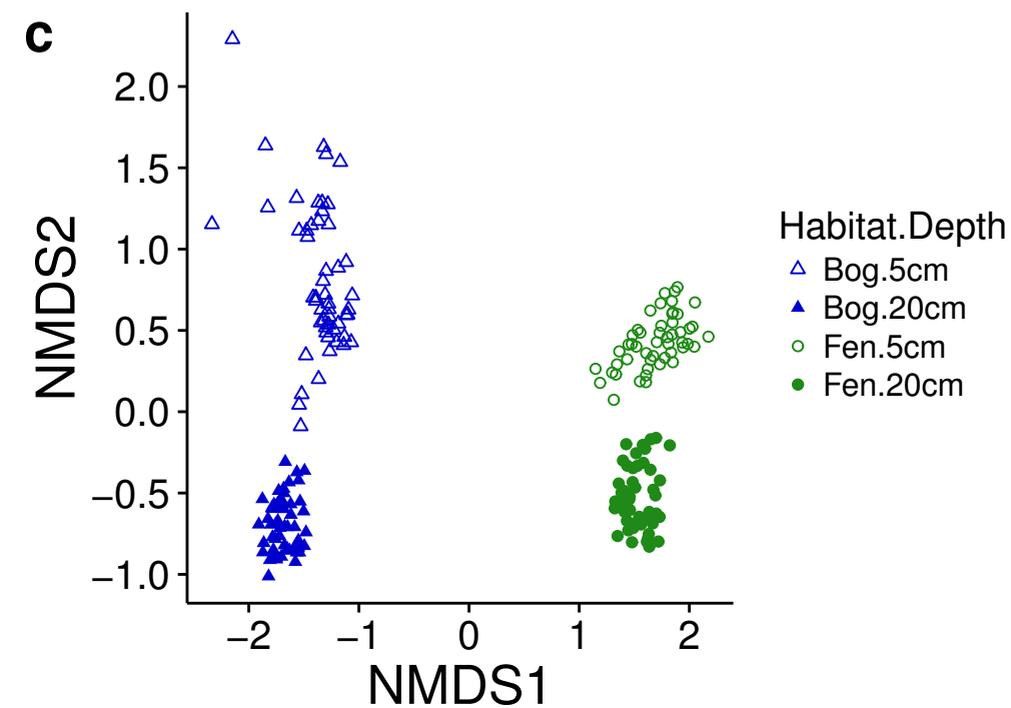
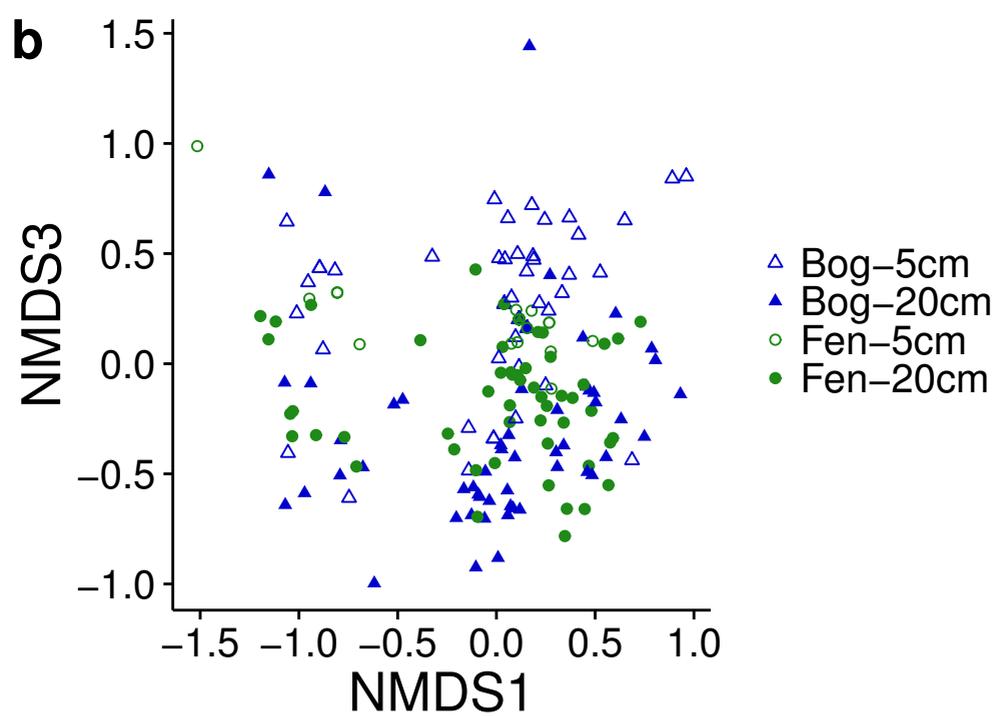
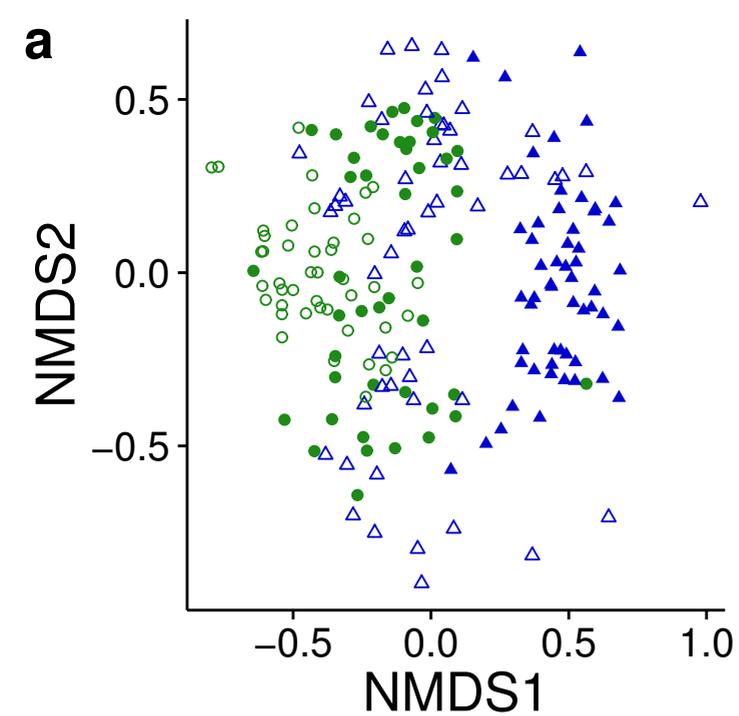
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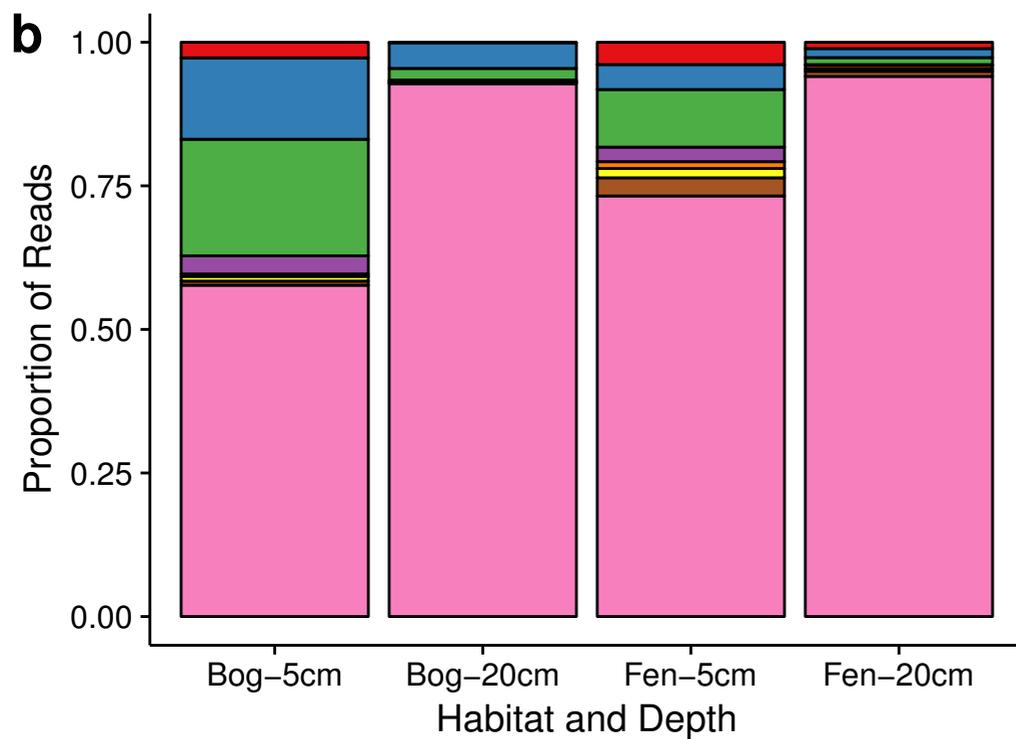
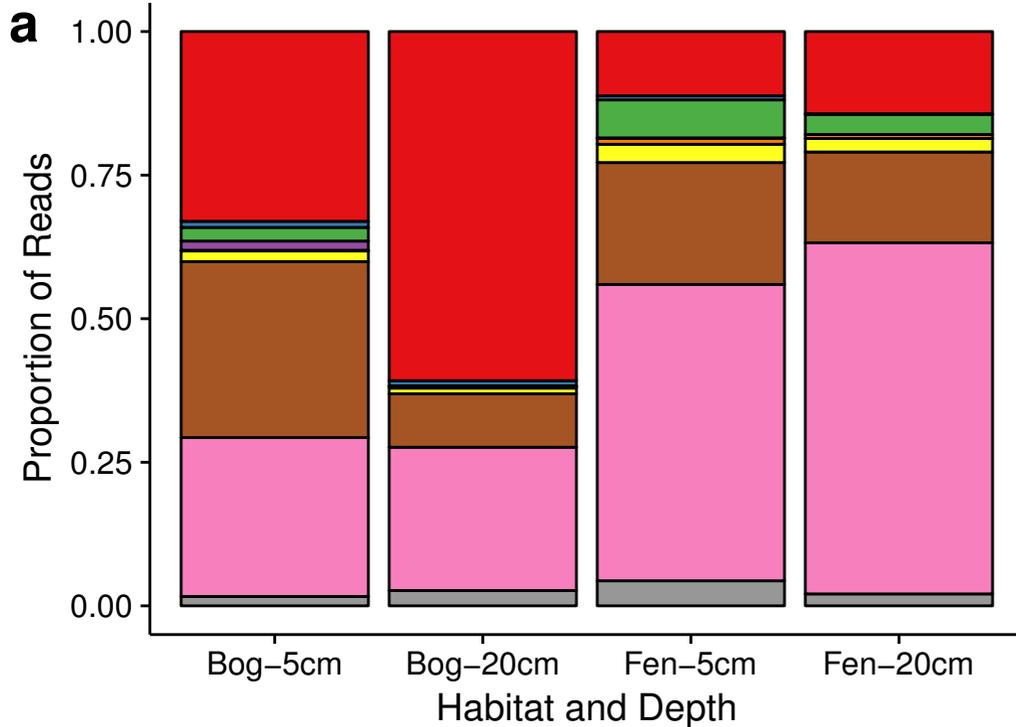
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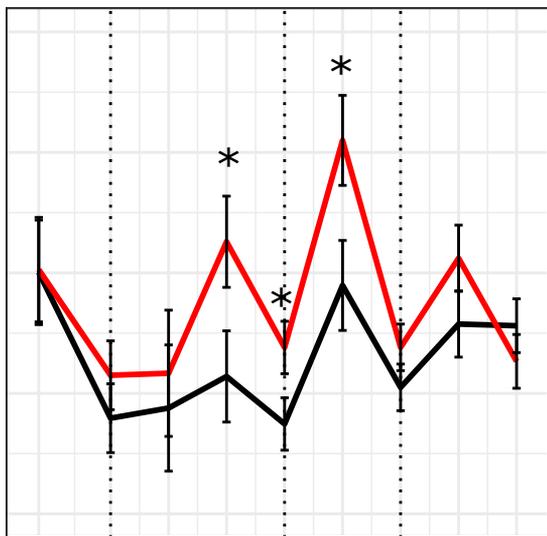
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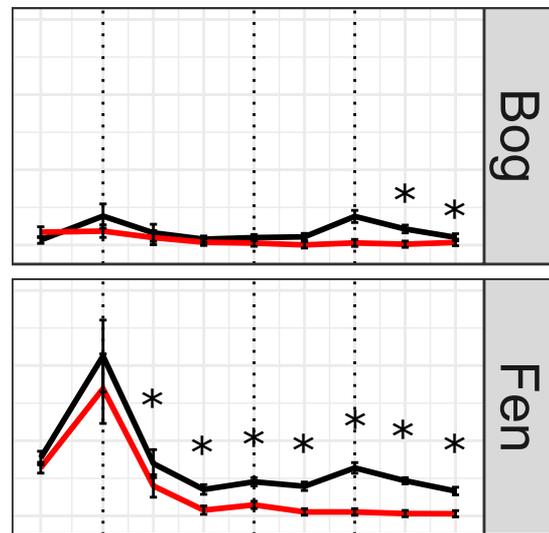




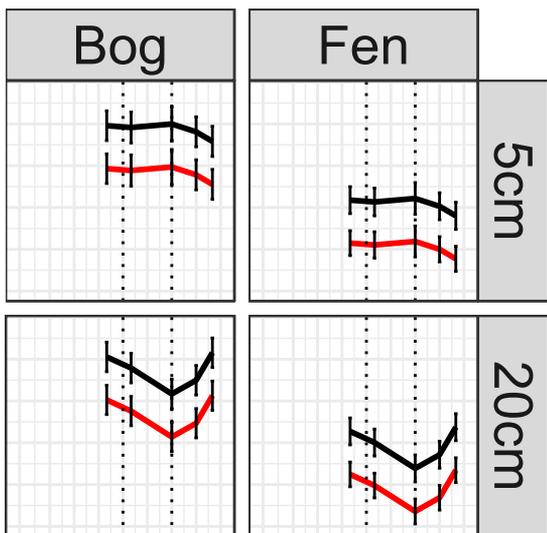
(a)



(b)



(c)



(d)

